HHS Public Access

Author manuscript

Dent Mater. Author manuscript; available in PMC 2017 July 18.

Published in final edited form as:

Dent Mater. 2015 October ; 31(10): 1245–1253. doi:10.1016/j.dental.2015.08.153.

Effect of crosslinking density of polymers and chemical structure of amine-containing monomers on the neutralization capacity of dentin adhesives

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Abstract

Objectives—Neutralization of the acidic micro-environment at the tooth/material interface is expected to provide enhanced durability for dental composite restorations. The objective of this study is to explore the effect of amine-containing monomer formulations and the crosslinking density of the resultant polymers on the neutralization capacity.

Materials and methods—The co-monomer system was varied systematically to obtain different proportions of Bisphenol A glycerolate dimethacrylate (BisGMA) and 2-hydroxyethyl methacrylate (HEMA), while maintaining a constant amount of amine-containing methacrylate monomer. A series of amine-containing monomers covering a range of p*K*a values were examined. Crosslinking density of formed copolymers was controlled by adjusting the weight content of the dimethacrylate monomer BisGMA. Lactic acid (LA) was used as a probe to analyze the effectiveness of the basic polymers to neutralize acid. The neutralization capacity of each amine-containing crosslinked copolymer was characterized by measuring pH as a function of time when the specimens were soaked in 1-mM LA solution, and the results were compared to the control formulations composed solely of BisGMA and HEMA. Polymer surfaces were examined using the methyl orange (MO) assay to quantify the amount of accessible amine groups.

Results—For each amine-containing crosslinked co-polymer, the neutralization capacity is enhanced by decreasing crosslinking density (e.g., by reducing BisGMA concentration in the formulation). In addition, more amine groups were accessible when crosslinking density was decreased. For different amine-containing polymers with the same BisGMA concentration, the neutralization capacity is higher when the amino monomers with higher p*K*a values were used in the formulations.

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Significance—This is the first time that the neutralization capacity based on crosslinked dental polymers has been studied. The information is important for future development of durable dentin adhesives.

Keywords

Amine monomer; Crosslinking density; Dentin adhesive; Neutralization; p*K*a

1. Introduction

Polymer-based composites have become the most common restorative material and are currently used more than twice as often as dental amalgam [1]. These resin composites fulfill many of the requirements for clinical restorative applications, including excellent esthetics. The durability of composite restorations does not, however, match that of dental amalgam [2–6]. The average clinical lifetime of composite resin restorations is just 5.7 years due to recurrent decay or fracture [7]. Recurrent decay has been linked to the failure of the bond between the tooth and composite and increased levels of the cariogenic bacteria *Streptococcus mutans* at the perimeter of these materials [8,9].

The composite is too viscous to bond directly to the tooth; a low-viscosity adhesive is used to connect the tooth to the composite. The adhesive bonds effectively to the acid-etched enamel, but bonding to dentin has been fraught with problems. In vitro and in vivo studies suggest that several factors inhibit the formation of a durable adhesive/dentin bond. One of the important factors is water sorption and hydrolysis of the adhesive polymers [10,11]. Water and saliva are always present in the mouth of healthy patients, and they are expected to penetrate into the free volume spaces between polymer chains. With water penetration, hydrolysis of ester groups in the polymer chains may occur, which shortens the clinical lifetime of polymer-based dental restoratives.

Another important factor is the polymer network structure, which is induced by photopolymerization of methacrylate monomers. Due to the rapid polymerization rate, after polymerization, highly crosslinked networks are formed, which are usually very heterogeneous due to the formation of highly crosslinked regions and loosely crosslinked regions [12–16]. The more heterogeneous a material, the more likely it is to have a significantly weaker structure in the regions of lower crosslinking, increasing the risk of premature failure.

The monomers used in dental restorative materials are particularly critical because polymerization of monomers produces the crosslinked matrix in the resultant polymers. Thus, monomer selection exerts considerable influence on the properties, durability and behavior of dentin adhesives in the wet, oral environment. Much attention and effort has been devoted to developing new adhesive monomers in order to enhance the lifetime of dental composite restorations [11,17–21].

Introducing neutralization capacity by using amine-containing monomers offers a promising approach to enhance hydrolysis resistance. The presence of water promotes the chemical hydrolysis of ester bonds in methacrylate materials [22–24]. This reaction might be

relatively slow at the neutral pH typical of saliva, but excursions in pH, caused by food or cariogenic bacteria that produce lactic acid, may lead to transient acid catalysis [25]. Degradation of methacrylate ester groups produces carboxylic acids, which contain the same functional group that is the culprit in lactic acid-induced decay. Degradation products from ester hydrolysis are more hydrophilic than the parent ester, which further enhances the local ingress of water and hydrolysis. With time, local domains of the methacrylate network may become sufficiently degraded and/or hydrophilic to permit access by esterases that greatly accelerate ester bond hydrolysis.

The chemical and enzymatic degradation of the methacrylate-based matrix could create a low pH environment at the composite/tooth interface because of the low p*K*a values of methacrylic acid and lactic acid, which are 4.66 and 3.86, respectively [20,26–31]. Furthermore, acidification of the oral microenvironment promotes demineralization of tooth structure at the margin of composite restorations. The increased surface roughness of the demineralized tooth surface creates additional opportunity for adhesion by biofilm, mainly salivary proteins and pioneer pathogenic bacteria, thereby accelerating the degradation process. We proposed that the pathogenic impact of biofilm at the margin of the composite restoration could be reduced by engineering novel dentin adhesives that neutralize the acidic microenvironment and resist biofilm attachment [32]. Integrating basic moieties with an appropriate p*K*a into methacrylate derivatives provides the opportunity to act as an acid-neutralizing proton sponge and/or buffer to protect against acid-induced degradation.

In our previous work, the neutralization capacity of amine-containing monomers was quantified in a water/ethanol co-solvent system and the effects of solvent environment on p*K*a were examined [33]. However, to our knowledge, there are no reports of neutralization capacity having been determined for amine-containing crosslinked polymers. As such, it is necessary to determine the correlation between the interrelated properties of neutralization capacity and crosslinking density/chemical structure of amine-containing monomers within crosslinked polymer systems.

The twofold objectives of this work are: (1) to study the crosslinking effect on the neutralization capacity of the polymer, and (2) to investigate the influence of the chemical structure of amine-containing monomers on the neutralization capacity. To our knowledge, this investigation marks the first study on the neutralization capacity of crosslinked dentin adhesives. The results provide important information to enable future development of durable dental restorative materials.

2. Materials and methods

2.1. Materials

Camphorquinone (CQ), ethyl-4-(dimethylamino) benzoate (EDMAB) and diphenyliodonium hexafluorophosphate (DPIHP) were used as a three-component-photoinitiator system. Bisphenol A glycerolate dimethacrylate (BisGMA) and 2-hydroxyethyl methacrylate (HEMA) were used as co-monomers. 2-(dimethylamino) ethyl methacry-late (DMAEMA), 2-(diisopropylamino) ethyl methacrylate (DIPAEMA), 2-(*tert*-butylamino) ethyl methacrylate (TBAEMA) and 2-*N*-morpholinoethyl methacrylate

(MEMA) were used as amine monomers. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. The chemical structures of monomers are shown in Table 1.

2.2. Preparation of adhesive formulations

The preparation of the adhesive formulations has been reported [34,35]. There are three control adhesive formulations, consisting of HEMA and BisGMA with a mass ratio of 45/55 (C0-55), 70/30 (C0-30), 85/15 (C0-15), which were used for comparison to the amine-containing experimental adhesive resins. For all experimental formulations, the weight content of amine monomer is 25 wt%. The methacrylate formulations are comprised of HEMA/amine monomer/BisGMA, and differ in that the content of BisGMA was decreased from 55, 30 to 15 wt% in order to examine the crosslinking effect on neutralization capacity at a constant amine concentration. Table 2 shows the adhesive formulations with weight percentage and molar ratio. A three-component photoinitiator system was used that contains CQ (0.5 wt%), EDMAB (0.5 wt%) and DPIHP (1.0 wt%). The resin mixtures were prepared in brown glass vials. The mixtures were shaken on an orbital shaker for two days to dissolve the initiators and form a homogeneous solution.

2.3. Real-time conversion

Real-time in situ monitoring of the photopolymerization of the adhesive formulations was performed using an infrared spectrometer (Spectrum 400 Fourier transform infrared spectrophotometer, Perkin-Elmer, Waltham, MA) at a resolution of 4 cm^{-1} [17,18,36,37]. One drop of adhesive solution was placed on the diamond crystal top-plate of an attenuated total reflectance (ATR) accessory (Pike, GladiATR, Pike Technology, Madison, WI) and covered with a mylar film. A 40-s-exposure to the commercial visible-light-polymerization unit (Spectrum 800®, Dentsply, Milford, DE, ~480–490 nm [38]), at an intensity of 550 mW cm^{-2}, was initiated after 50 spectra had been recorded. Real-time IR spectra were recorded continuously for 600-s after light curing began. A time-resolved spectrum collector (Spectrum TimeBase, Perkin-Elmer) was used for continuous and automatic collection of spectra during polymerization. Three replicates were obtained for each adhesive formulation.

2.4. Preparation of polymer samples

Disc samples with a thickness of 1 mm and a diameter of 4 mm were prepared by injecting the adhesive formulations into hermetic lids (TA instruments, T 120110, USA) and each covered with a round glass coverslip (Ted pella, Inc., Prod no. 26023). Ten specimens were prepared for each formulation. The samples were light polymerized with a 40-s exposure to the commercial visible-light polymerization unit (Spectrum®, Dentsply, Milford, DE) at an intensity of 550 mW cm^{-2}. The polymerized samples were stored in the dark at room temperature for two days to provide adequate time for post-cure polymerization. The resultant disc samples were used for pre-wash, neutralization and water sorption experiments.

2.5. Prewash of disc samples

Five disc samples for each formulation were placed in a flask containing 100 mL deionized water and shaken at 37 °C for 5 days. The water was changed daily. At day 6, the solution was removed. The specimens were rinsed with water and then dried in a vacuum oven at 37 °C for 15 days.

2.6. Neutralization experiments and pH measurements

A 2 mL volume of 1-mM lactic acid (pH 3.5) was added into the vial and mixed with 0.2 g of disc samples. The vials containing the control and experimental solutions were placed on the shaker at room temperature and the pH was measured at fixed time intervals. The pH measurements were performed with a Fisher Scientific (Waltham, MA, USA) Accumet Research AR25 pH meter equipped with a micro-probe. Calibration was done using commercial buffer standards (Fisher Scientific, pH 4.01, 7.00, and 10.01)

2.7. Water sorption

The water sorption protocol has been reported previously [39]. After prewash, the dry disc samples ($m_{1\ \mathrm{dry}}$) were then immersed in deionized water and stored at room temperature. At fixed time intervals (6, 24, 48, 72, 120, 168 and 240 h), the polymer samples were retrieved, blotted dry to remove excess liquid, weighed ($m_{2\ \mathrm{wet}}$) and re-immersed in the water. The value (%) for mass change due to water sorption was calculated as follows:Mass

$$\text{change}\,(\%) = 100\frac{m_{2\ \mathrm{wet}} - m_{1\ \mathrm{dry}}}{m_{1\ \mathrm{dry}}}$$

2.8. Methyl orange (MO) assay to detect the surface amine groups

The methyl orange assay was performed as reported previously [40,41]. A 0.2 wt% methyl orange (Fisher Scientific) stock solution was prepared using deionized water. This solution was diluted to 0.05 wt% with 0.1 M NaH_2PO_4 solution (Fisher Scientific) to prepare an acidic methyl orange solution, such that the final buffer concentration was 0.075 M NaH_2PO_4 and solution pH was 4.4.

Samples were prepared in 96-well plates. To make the polymer coating, 60 μL of resin formulation was added to each well. Each resin formulation was analyzed in triplicate. Polymerization and curing in the 96-well plates was performed using oxygen-depleted conditions in which the plate was placed in a sealed enclosure and purged with nitrogen for 10 min. Monomer resins were cured in a curing chamber (Triad® 2000™, visible light cure system, Dentsply, York, PA) at an intensity of 50 mW cm^{-2} with a 16-min total exposure time in which the plate was rotated by 45 degrees every 2 min to ensure complete polymerization in all wells. The plate was left in the dark for post polymerization for 48 h. At 48 h 0.3 mL of water was added to each well. The water was changed twice during each week to remove leachable components. After 5 weeks prewash, water was removed and polymers were rinsed under a stream of deionized water for approximately 10–30 s, and excess water was removed by blotting samples with a Kimwipe. 50 μL of acidic MO solution was added in each well and incubated for 5 h. The MO solution was decanted and the wells were washed with deionized water 10 times; excess water was removed by blotting samples with a Kimwipe. 200 mL 0.1 M Na_2CO_3 (Fisher Scientific) solution, pH 11.0 was

added in each well and incubated for 72 h. The final solution in each well was extracted; the solution was diluted 40 fold, and transferred to a new plate for analysis.

The 96-well plate was read using the plate reader (CYTATION 3 imaging reader, BioTek Instruments, Inc., Minooski, Vermont, USA) in bottom-read absorbance mode. Methyl orange concentration was determined from measuring absorbance at 465 nm and comparing the data to a standard calibration curve. The standard curve was linear over the concentration range investigated. Finally, the accessible amine density on the polymer surface was calculated based on the surface area of a well in a 96-well plate, which was calculated to be 0.33 cm^2.

2.9. Statistical analysis

The results were analyzed statistically using analysis of variance (ANOVA), together with Tukey's test at α= 0.05 (Microcal Origin Version 8.0, Microcal Software Inc., Northampton, MA).

3. Results

Fig. 1 and Table 3 show the comparative results of pH versus time plots for different amine-containing polymers with different crosslinker concentration. Note that samples identified with the prefix C0 are controls, and they do not contain any amine. Fig. 1A shows the results with 55 wt% BisGMA. The following is the neutralization capacity from highest to lowest: TBAEMA, DMAEMA, DIPAEMA, MEMA, C0-BisGMA55 (no amine). Fig. 1B shows the pH results for the polymers containing 30 wt% BisGMA. The neutralization capacity from highest to lowest for amine-containing polymers at 30 wt% BisGMA is as follows: TBAEMA, DMAEMA, DIPAEMA, MEMA. Fig. 1C shows the pH results for the series containing 15 wt% BisGMA. The neutralization capacity from highest to lowest for amine-containing polymers at 15 wt% BisGMA is as follows: TBAEMA, DIPAEMA, DMAEMA, MEMA. The trend is the same when there is 55 or 30 wt% of BisGMA, but DMAEMA showed an unexpected result when BisGMA was reduced to 15 wt%. The final pH values after 60 days are presented in Table 3.

Fig. 2 shows the water sorption results for the three control formulations, TBAEMA and the MEMA-containing polymers. Prewash and drying was done before the water sorption experiments, thus, the mass change values reflect solely the water sorption values. Water sorption increased with decreasing BisGMA concentration for all the formulations. The water sorption values increased and were 9.8, 17.8 and 29.0% for control formulations with 55, 30 and 15 wt% BisGMA (Table 3), respectively. For TBAEMA-containing polymers, the water sorption was 8.6, 14.3 and 17.2% with decreasing BisGMA concentration (Table 3), respectively. The water sorption values for MEMA-containing polymers with decreasing BisGMA content were 8.3, 18.6 and 30.6% (Table 3), respectively.

The DC results for the control, the TBAEMA-containing and the MEMA-containing formulations are presented in Table 3. At 10 min the DC was 68.7, 68.5 and 65.6% for control formulations with 55, 30 and 15 wt% BisGMA concentration, respectively. For TBAEMA-containing resins, the DC increased and was 71.8, 81.0 and 82.2% with

decreasing BisGMA concentration, respectively. The DC values for MEMA-containing resins with decreasing BisGMA concentration was 75.0, 82.6 and 81.9%, respectively.

Fig. 3 shows the results of accessible amine density on the polymer surfaces, calculated from MO concentration (Table 3) based on desorption of MO molecules from amine-containing polymer surfaces per unit area of a well in a 96-well plate (0.33 cm^2). For control formulations, the accessible amine density measurement was performed to establish background and yields a similar result for each formulation: 3.8×10^{-8} (C0-55), 3.8×10^{-8} (C0-30) and 3.5×10^{-8} (C0-15) mol cm^{-2}. For TBAEMA-containing resins, the accessible amine densities increased by at least 10 fold above background and were 31.7×10^{-8}, 59.7×10^{-8} and 74.2×10^{-8} mol cm^{-2} with decreasing BisGMA concentration. The accessible amine densities for MEMA-containing resins also increased substantially and were 24.4×10^{-8}, 40.2×10^{-8} and 63.3×10^{-8} mol cm^{-2} with decreasing BisGMA.

4. Discussion

The pH results shown in Table 3 indicate that neutralization capacity could be increased by decreasing crosslinking density (e.g., by reducing the crosslinker concentration used in the adhesive formulation). The only exception is the DMAEMA-containing polymer series, as shown in Fig. 1A, in which decreased BisGMA content from 30 to 15 wt% does not result in a greater uptake of protons, as the final pH values after 60 days neutralization are 5.75 and 5.22, respectively. That is, the neutralization capacity was not enhanced by decreasing crosslinker concentration. This result could be attributed to the relative hydrophilicity of DMAEMA (Log *P*: 0.99, calculated by *ChemBioDraw Ultra 12.0*) compared to DIPAEMA (Log *P*: 2.3) and TBAEMA (Log *P*: 1.49) (Note: Octanol–water partition coefficient log *P* is used as a measure of molecular hydrophobicity [42]. A partition coefficient is the ratio of concentrations of a compound in the two phases of a mixture of two immiscible liquids at equilibrium. Hence both the partition and distribution coefficient are measures of the hydrophilic (“water-loving”) or hydrophobic (“water-fearing”) nature of a chemical substance.) During polymerization, DMAEMA might not become crosslinked as effectively into the polymer network when the crosslinker concentration is decreased. Unincorporated amine-containing monomers and low molecular-weight oligomers would be removed during the prewash process, thereby decreasing the neutralization capacity at the lowest crosslinker concentration. It should be noted that the pH value for MEMA-15 also was not increased from MEMA-30 (Table 3), and there is no significant difference between them, because MEMA also is more hydrophilic (Log *P*: 0.59). Therefore, for formulations containing more hydrophilic amine monomers at lower crosslinker concentrations (such as 15 wt%), leaching of un-polymerized monomers and oligomers during the prewash process may influence the neutralization results. Nonetheless, a clear neutralization trend for crosslinked polymers still can be derived from Fig. 1 and Table 3, which is that neutralization capacity is increased with decreasing crosslinker concentration, likely due to increased ingress of water.

The slight pH changes during 60 days storage for control (C0-55) samples can be explained by the presence of 0.5 wt% EDMAB (the amine co-initiator in the system). The enhanced neutralization capacity for amine-containing polymers could be a reflection of increased porosity or decreased density, which permits more water sorption. In this way, amine groups

would be more readily accessible for protonation. In order to test this hypothesis, water sorption experiments were conducted on the control, TBAEMA and MEMA-containing crosslinking polymers. The results of the water sorption experiment indicate that more water was absorbed into the polymer when the crosslinker concentration was decreased. TBAEMA is more hydrophobic than HEMA and MEMA, and as such there is less water sorption into these polymers than the control and MEMA-containing formulations with the same BisGMA concentration. In addition, the DC results show monomer-to-polymer conversion can be increased in the presence of amine monomers, because amine monomers might serve as co-initiators.

It is noted that the disc samples with a thickness of 1 mm in this study are not representative of the clinical application of adhesives. In the clinical application, the adhesive layer could vary from tens of microns to hundreds of microns depending on the adhesive viscosity and number of layers applied to the dentin substrate. It is expected that as a result of the reduced thickness the timeframe for the amine-containing copolymers to buffer could be much shorter. The purpose of this investigation is to explore the relationship between the crosslinking density and the neutralization capacity using the standard experimental system. Thicker samples were used because the sample preparation is more straightforward and it is easier to handle the thicker samples. In addition, the sample preparation was of consistent with the requirements for water sorption study and MO assay.

We also determined that the storage stability for DMAEMA and DIPAEMA-containing formulations is poor. All the DMAEMA and DIPAEMA-containing resin formulations auto-polymerized over one week when the liquid resins were stored in the dark. This instability might be caused by the reaction between amine monomer and iodonium salt, because iodonium salt is an oxidizing agent. TBAEMA- and MEMA-containing formulations are quite stable and no auto-polymerization was detected after several months of storage. Based on these observations, DC and water sorption experiments were conducted only for the control, TBAEMA- and MEMA-containing formulations. In addition, all the amine-containing specimens appear yellow under both dry and wet conditions except MEMA-containing specimens. The MEMA-containing specimens appear yellow under dry conditions, but dark-green under wet conditions (soaking in water). The yellow color seems stable during long-term storage (six month storage under dry condition). We have not found significant color changes due to long-term oxidation.

By comparing different amine-containing polymers comprised of equivalent crosslinker content, as shown in Fig. 1 and Table 3, TBAEMA-containing polymers have the highest neutralization capacity and MEMA-containing polymers have the lowest neutralization capacity. At equivalent crosslinker content, it is anticipated that the polymer networks would have similar crosslinking density. The differences in neutralization capacity might be caused by different chemical potentials of each polymerized system, which would affect the rate of water infiltration and the ability of the amino groups to become protonated and alter the pH (As reported [43,44], $\mu = -2.3RT\mathrm{p}k\alpha$, where μ is the chemical potential). Although the p*K*a values for crosslinked polymers cannot be measured directly, the p*K*a values for the amine monomer have been examined in our previous work [33]. The p*K*a values for TBAEMA, DMAEMA, and MEMA are 9.7, 8.6 and 6.2, respectively (DIPAEMA was not examined in

our previous work because of insufficient solubility in the co-solvent system). Thus, at the same crosslinking density, the p*K*a values for these amine-containing polymers likely share the same tendency as the monomers. Therefore, the chemical potential for protonation is more negative for polymers with higher p*K*a values. This could be the reason for greater neutralization capacity when amine monomers with higher p*K*a values are compared at equivalent crosslinker concentrations.

Neutralization capacity reports on the effectiveness of the initial uptake of protons into the polymer, but in the oral environment the ability of the amines to buffer against pH excursions is also important. Although the MEMA-containing polymers demonstrate less neutralization capacity compared to the other amines (Fig. 1), the lower p*K*a of MEMA (6.2) results in the most effective buffering in the relevant pH range and has a less alkaline end point. Consequently, the MEMA-containing polymer has the ability to act as a proton sponge and also provide buffering to potentially offer better protection against acid-catalyzed hydrolysis, particularly in regions where the crosslinking density is lower. The balance of lower crosslink density and suitable mechanical properties must be considered in the design and development of these amine-containing adhesive formulations. There are ongoing investigations to determine the dynamic mechanical properties of these experimental adhesives.

In order to enhance the durability and extend the clinical lifetime of dentin adhesives, further modification of the surface of dentin adhesives could be performed, such as grafting of functional peptides or inhibitors. Therefore, the concentration and availability of amine groups at the polymer surface and within the bulk polymer are critical parameters. The MO assay was used to quantify the accessible amine groups on the surface of polymers. The results in Fig. 3 indicate approximately twice as many amine groups are accessible when the concentration of crosslinker is decreased from 55 to 30 wt%. This correlates with the results of the neutralization capacity measurements, further indicating that inclusion of amine moieties in the polymer formulation generates an adhesive that can neutralize acid.

5. Conclusion

Neutralization capacity of crosslinking polymers was studied by adjusting the crosslinking density and employing different amine-containing monomers. The results indicate neutralization capacity is enhanced by decreasing the crosslinking density of the polymers. Greater neutralization capacity is obtained by introducing amine monomers with higher p*K*a values, but incorporation of monomers with physiologically relevant p*K*a values provides buffering to protect against acid-induced degradation, suggesting that a combination of amines may provide maximum utility in practice.

Acknowledgments

This investigation was supported by research grant: R01 DE022054 and DE022054-04S1 from the National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD 20892.

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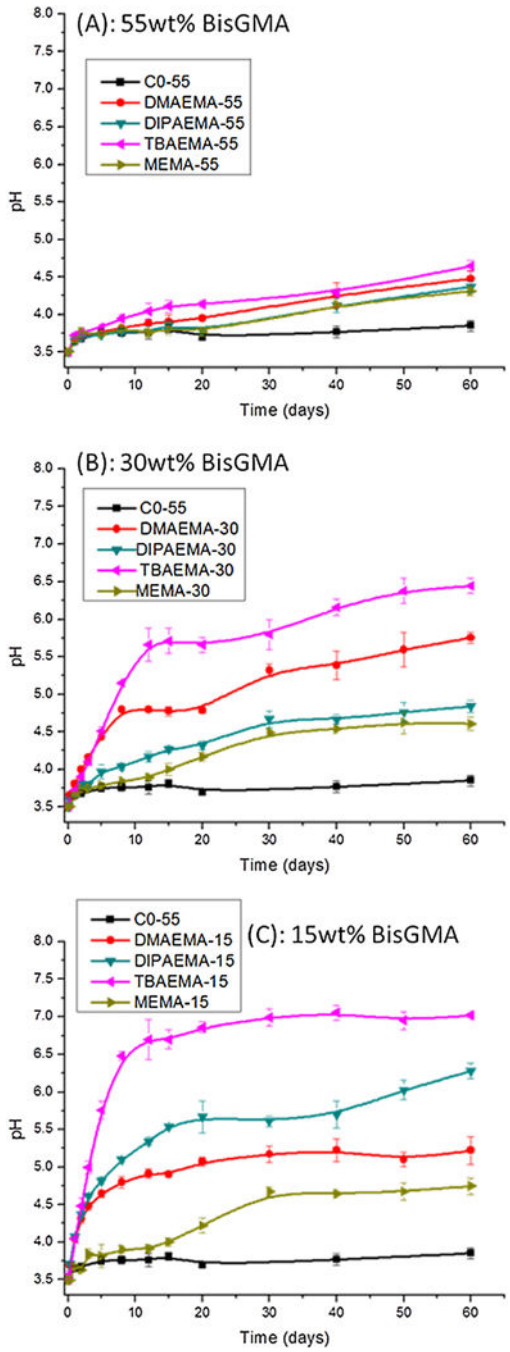


Fig. 1.

Plots showing the ability of different amine-containing polymers to neutralize LA. Each plot shows the pH of a set of amine-containing polymers with equivalent BisGMA concentration as a function of incubation time. (A) 55 wt% BisGMA; (B) 30 wt% BisGMA; (C) 15 wt% BisGMA. The control formulation (C0-55), which lacks amine moieties, is shown in black on each plot for comparison.

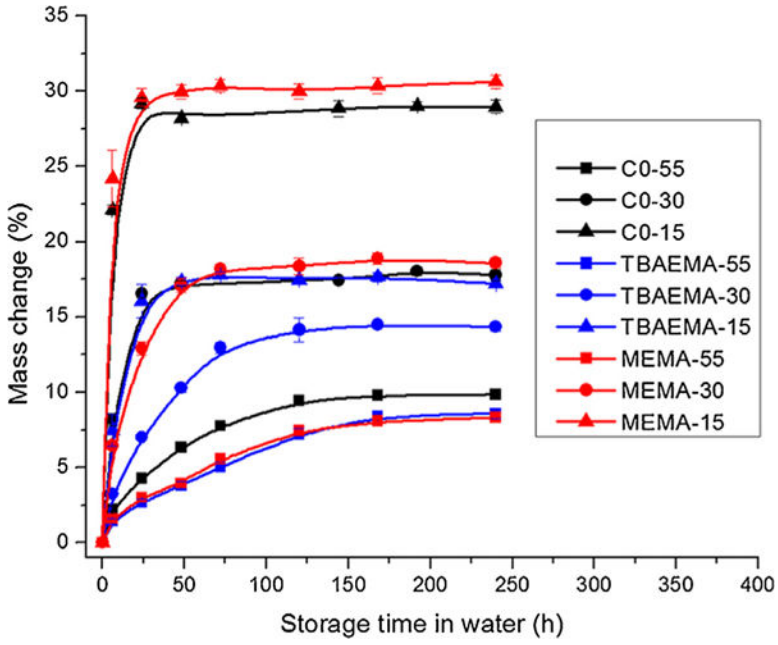


Fig. 2.

Water sorption analysis. The change in mass of each crosslinked polymer is plotted as a function of storage time in water.

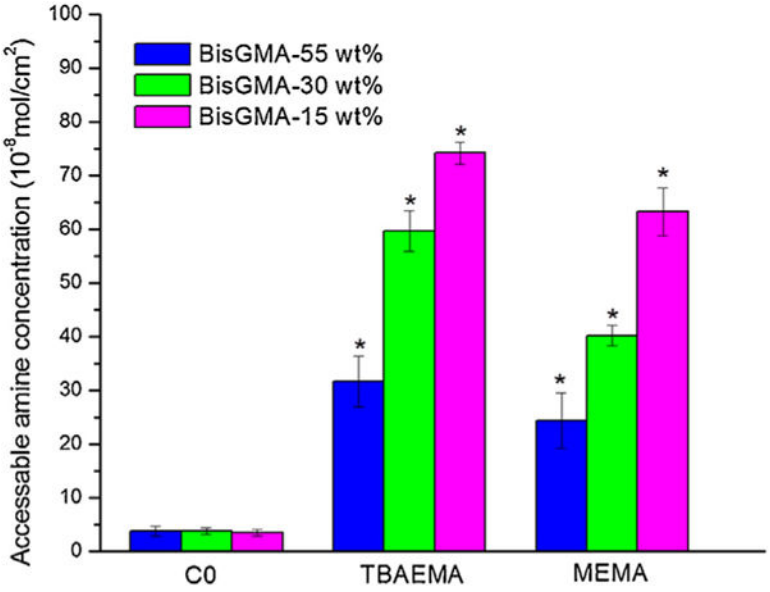


Fig. 3.

Characterization of accessible amine content on the polymer surfaces. The concentration of amine was calculated based on the results of the MO assay divided by the surface area of the well in the 96-well plate. * Significant ($p < 0.05$) difference from control formulations.

Table 1

Chemical structures of monomers used in neutralization experiments.

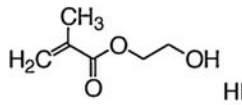
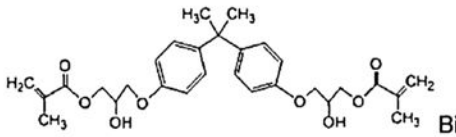
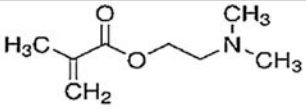
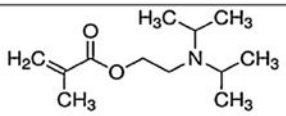
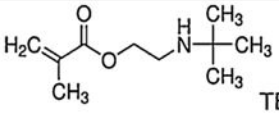
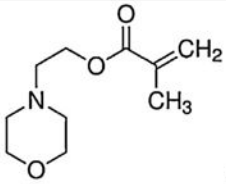
 HEMA	 BisGMA
Amine monomers:	
 DMAEMA, pKa 8.6	 DIPAEMA
 TBAEMA, pKa 9.7	 MEMA, pKa 6.2

Table 2

Adhesive formulation used in neutralization experiments.

Samples	HEMA (wt%)	Amine monomer (wt%)	BisGMA (wt%)	Mole of amine/gram (10^{-3})	Molar ratio[a]
C0-55	45	0	55	0	3.23/1[b]
C0-30	70	0	30	0	9.12/1
C0-15	85	0	15	0	22.52/1
DMAEMA-55	20	25	55	1.6	0.97/1/0.67
DMAEMA-30	45	25	30	1.6	2.18/1/0.37
DMAEMA-15	60	25	15	1.6	2.90/1/0.18
DIPAEMA-55	20	25	55	1.2	1.32/1/0.92
DIPAEMA-30	45	25	30	1.2	2.96/1/0.50
DIPAEMA-15	60	25	15	1.2	3.94/1/0.25
TBAEMA-55	20	25	55	1.4	1.14/1/0.79
TBAEMA-30	45	25	30	1.4	2.56/1/0.43
TBAEMA-15	60	25	15	1.4	3.41/1/0.22
MEMA-55	20	25	55	1.3	1.23/1/0.86
MEMA-30	45	25	30	1.3	2.77/1/0.47
MEMA-15	60	25	15	1.3	3.69/1/0.23

[a] Molar ratio of the ternary monomer formulations: HEMA/amine/BisGMA.

[b] Molar ratio of the HEMA/BisGMA.

Table 3

Results summary in neutralization experiments.

Samples	Sample pH[a]	Water sorption (wt%)[b]	DC (%)[c]	MO concentration (μg/mL)	Accessible amine density (10^{-8} mol cm^{-2})[d]
C0-55	3.85 ± 0.07	9.8 ± 0.1[f,h]	68.7 ± 0.1	20.4 ± 4.9[h]	3.8 ± 0.9[h]
C0-30		17.8 ± 0.4[e,f,h]	68.5 ± 0.4	20.7 ± 3.0[h]	3.8 ± 0.5[h]
C0-15		29.0 ± 0.5[e,f,h]	65.6 ± 0.3[e,f]	18.9 ± 3.6[h]	3.5 ± 0.7[h]
DMAEMA-55	4.48 ± 0.11[e,f]				
DMAEMA-30	5.75 ± 0.07[e,f,h]				
DMAEMA-15	5.22 ± 0.18[e,f]				
DIPAEMA-55	4.37 ± 0.07[e,f]				
DIPAEMA-30	4.84 ± 0.08[e,f,h]				
DIPAEMA-15	6.28 ± 0.10[e,f]				
TBAEMA-55	4.65 ± 0.07[e,f,h]	8.6 ± 0.3[e,f]	71.8 ± 0.4[e,f]	172.0 ± 25.6[e,f,h]	31.7 ± 4.7[e,f,h]
TBAEMA-30	6.45 ± 0.10[e,f]	14.3 ± 0.3[e,f,h]	81.0 ± 0.2[e,f]	323.8 ± 20.8[e,f,h]	59.7 ± 3.8[e,f,h]
TBAEMA-15	7.02 ± 0.03[e,f]	17.2 ± 0.3 [e,f,h]	82.2 ± 0.3[e,f]	402.7 ± 22.7[e,f,h]	74.2 ± 2.0[e,f,h]
MEMA-55	4.31 ± 0.06[e,f]	8.3 ± 0.1[e,f]	75.0 ± 0.5[e,f]	132.5 ± 28.1[e,f,h]	24.4 ± 5.2[e,f,h]
MEMA-30	4.61 ± 0.09[e,g,h]	18.6 ± 0.4[e,f,h]	82.6 ± 0.4[e,g]	218.3 ± 10.4[e,f,h]	40.2 ± 1.9[e,f,h]
MEMA-15	4.72 ± 0.11[e,g,h]	30.6 ± 0.5[e,g,h]	81.9 ± 0.4[e,g]	343.5 ± 24.4[e,g,h]	63.3 ± 4.5[e,g,h]

[a] Values were obtained after 60 day incubation in 1 mM LA, pH 3.50.

[b] Values were obtained after 240 h incubation in water.

[c] Obtained 600 s after light curing began.

[d] The accessible amine concentration was calculated based on the surface area of a well in a 96-well plate being 0.33 cm^2.

[e] Significant ($p < 0.05$) difference from C0-55.

[f] Significant ($p < 0.05$) difference from formulations with the same amine monomers.

[g] There is no significant ($p < 0.05$) difference between MEMA-30 and MEMA-15.

[h] Significant ($p < 0.05$) difference from formulations containing different amine with the same BisGMA content.